

# Bioactive Organic-Inorganic Composite Monolith Derived from Poly Vinyl Trimethoxy Silane Using Sol- Gel Process

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## Abstract

Homogeneous and crack-free with micron pores organic-inorganic composite monoliths in a binary system of Ca and Si, were prepared by means of a sol-gel route starting from polymerized vinyltrimethoxysilane, about 20-mers, and an alcoholic solutions of calcium acetate. The surfaces of the specimens were characterized using XRD, FT-IR, SEM and EDX analysis. In vitro bioactivity tests were performed in SBF and showed that all composite monoliths produced were bioactive, indicating that the composite monolith with composition VTMS:H<sub>2</sub>O:i-PrOH:Ca(OAc)<sub>2</sub> = 1:6:6:0.2 (mol) can be suitable for the molecular design and fabrication of bioactive tissue engineered scaffolds.

## Keywords

*Organic-Inorganic Composite; Monolith; Sol-Gel Process; Bioactivity*

## Introduction

The sol-gel process is a chemical synthesis technique for the preparation of glasses and ceramics. The preparation of monolithic glasses, glass fibres, pure fine powders and other ceramic materials from metal alkoxides or metal salts by the gel-method has been reported since late 1970s by Hench et. al. and Babonneau et. al.. Sol-gel processing has been intensely studied as an alternative method for preparing ceramics and glasses for a wide variety of applications, including the field of bioceramics. The high bioactivity of the sol-gel derived materials is related to the textural features of the gels, i.e., pore size and pore volume associated with the large surface area, higher rate of dissolution, and the negative surface charge (Pereira et. al and Nasr-Esfahani et. al.).

In the early 1990s, Kokubo and his co-workers found that glass or glass ceramics with lime and silica as major ingredients formed a silica gel layer on their surface in the body environment, and that the layer served as nucleating sites for crystallization of

calcium-deficient apatite, which formed a bond with bone. After that, the sol-gel processing method was used to produce powder and monolithic samples of the gel the CaO-SiO<sub>2</sub> glasses for use in tissue engineering applications (Saravanapavan et. al.). Monolithic gels are potentially of interest because complex shapes may be formed at room temperature and considered at rather low temperatures without melting. Monoliths are defined as bulk gels (smallest dimension  $\geq 1$  mm) cast to shape and processed without cracking (Brinker et. al.). The main reason for difficulty in making bulk glass consists in the fact that cracks and fractures are often generated when a bulk wet gel body containing solvents is dried. One of the methods to avoid occurrence of cracks and fracture during drying of gels and subsequent heating for gel to glass conversion is when an organic polymer is incorporated. The solutions Si(OCH<sub>3</sub>)<sub>4</sub>-H<sub>2</sub>O-HNO<sub>3</sub>-polymer containing poly(acrylic acid), poly(sodium styrenesulfonate) or poly(ethylene oxide) as organic polymer component are used to produce silica gels consisting of interconnected micrometer size silica networks and pores (Nakanishi et. al.). This structure results from the concurrent occurrence of phase separation of the solution and sol-gel transition of silica-rich phase.

Recently, organically modified sol-gel-derived metal oxides are attracted attention because of their unique properties, such as low elastic modulus and deformability (Mackenzie et. al. and Margarita et. al.). In these hybrid materials, organic components are chemically incorporated into inorganic networks at nanometer level. It is highly probable that organic polymers are bioactive if incorporating silanol groups and calcium ions since they are believed to play important roles in bioactivity. Organic-inorganic composite coatings have been prepared, starting from vinyltrimethoxysilane, by sol-gel processing and their

bioactivity has been confirmed (Tsuru et. al.). It is expected that monoliths of such bioactive gels may widen the biomedical use of organic polymer materials. In the present study, we prepared such monoliths in a modified procedure, studied optimum conditions to develop homogeneous, smooth, porous structure and crack-free, and examined changes in bioactivity of the monoliths with respect to Ca contents. Our goal was to obtain a more versatile compositional system for the molecular design and fabrication of bioactive multifunctional tissue-engineered scaffolds.

## Experimental

### Materials

Starting materials used in this preparation were analytical grade Vinyltrimethoxysilane (VTMS, Merck), tertiary butyl peroxide (Merck),  $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$  (Merck), nitric acid (Merck), isopropanol (*i*-PrOH, Merck). All materials were used as received without further purification.

Mixture of *i*-PrOH and double distilled water was used as solvent with molar ratio 1:1. 2N nitric acid ( $\text{HNO}_3$ ) was used to catalyze the PVTMS hydrolysis during the sol preparation along with molar ratios  $\text{H}_2\text{O}/\text{VTMS}$  of 6:1 (i.e. excess water) and volume (v) ratios  $\text{H}_2\text{O}/\text{HNO}_3$  of 6:1. Table 1 shows the final mixing ratios of the chemicals.

TABLE 1 THE COMPOSITIONS OF ORGANIC-INORGANIC COMPOSITE GELS (MOL) AND THEIR GELATION TIMES (DAYS)

Gel	Mixing ratios (mol)		Gelation time (day)	Description of monolith
	VTMS	$\text{Ca}(\text{CH}_3\text{COO})_2$		
M1	1.0	0	3	Transparent, homogeneous
M2	1.0	0.1	2	Opaque, homogeneous
M3	1.0	0.2	2	Opaque, homogeneous
M4	1.0	0.3	2	Opaque, heterogeneous

### Preparation of Poly Vinyltrimethoxysilane (PVTMS)

Vinyltrimethoxysilane (6 g, 0.04 mol) was polymerized to 20-mers with tertiary butyl peroxide (0.37 ml, 5 mol%) as an initiator using refluxing for 2 h at 150°C in flowing nitrogen.

### Mixing Order

2N  $\text{HNO}_3$  was added to the mixture of water and *i*-PrOH and gently stirred for 5 min. PVTMS was then

added in small amount over a 30-min period. This mixture has been maintained for a half hour to ensure complete hydrolysis and the progression of condensation. The calcium acetate was then added to this mixture and allowed to dissolve. Pouring and casting was achieved an hour later.

### Monolith Synthesis

Fig. 1 schematically illustrates the process followed to obtain cylindrical monoliths. The sol was prepared at room temperature in Pyrex glass beakers using the mixing order described above, and then cast into Teflon mould with diameter 25 mm and thickness up to 30 mm for gelation. Both aging and drying of wet gels were conducted in a programmable oven. Aging of the gels took place at 60°C for 72 h. The drying of the gels was then conducted reaching a maximum temperature of 320°C for 1 h in flowing nitrogen. The drying schedule used a slow ramping rate (1°C/min) to obtain crack free monoliths.

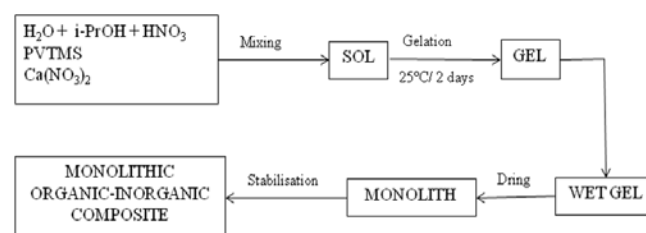


FIG. 1 SCHEMATIC ILLUSTRATION OF THE SOL-GEL PROCESS FOLLOWED FOR THE PREPARATION OF ORGANIC-INORGANIC COMPOSITE MONOLITHS

Approximately 10% shrinkage was observed during aging and drying. The organic-inorganic composite monoliths produced were cylindrical, with dimensions 22–23 mm in diameter and 4–5 mm in height without cracks (Fig. 2). Synthesized monolith in this process is like a yellow slice.



FIG. 2 ORGANIC-INORGANIC COMPOSITE MONOLITHS PRODUCED

### Characterization

TGA curves were recorded under dynamic argon-flow at a heating rate of 10°C/min on crushed bulk specimens from room temperature to 400°C. A Setaram1640 model was used with  $\text{Al}_2\text{O}_3$  as the

reference material. Scanning electron microscope (Vega (II) Tescan) was used to determine the morphology of monolith. The same instrument was also used to perform electron diffraction spectroscopy (EDS) quantitative analysis of monolith. The surfaces of the monoliths made were analysed using a powder diffractometer (Philips Xpert-MPD). XRD measurements were performed by the step scanning method with CuK $\alpha$  radiation. The instrument was operated at 40 kV and 40 mA with a 0.040° 2 $\theta$  step and a count rate of 30 s/step, the 2 $\theta$  values ranging from 10° to 50°. The formation of the apatite layer was analyzed using Fourier transform infrared spectroscopy (FTIR) (Bomem, MB-100) in the range 2000–600 cm<sup>-1</sup>.

### *In Vitro Bioactivity Testing*

Simulated body fluid (SBF) soaking was used to evaluate the in vitro bioactivity of monolith samples. The composition of this buffer, described by Kokubo has an ionic composition similar to that of human blood plasma. SBF is reported to produce the same type of hydroxyapatite layers in vitro, as it would form on the glass surface in vivo.

The samples were immersed in simulated body fluid and all the immersed samples were hold in a water bath at 37°C for 30 days.

## Results and Discussion

### *Gel Properties*

Table 1 describes the appearance of typical monolithic gels. Addition of calcium acetate content decreased homogeneity and M3 with the greatest calcium acetate content led to a homogeneous monolithic gel.

The aged gel, termed WET, contains the most amount of solvent while the dried samples, termed DRY, contain hardly any solvent. Fig. 3 shows the TGA curves of the WET and DRY gels. The WET sample showed an initial endothermic process in the range of 130–160°C, which was attributed to a residual loss of water and alcohol, corresponding to a weight loss of 4–5%. A second endothermic process occurred at a temperature between 220 and 300°C, with a weight loss of 5–6%; then, the weight remained constant up to 400°C. This last process was attributed to the loss of the carbonization or the combustion of organic compounds and decomposition of acetates used in the sol preparation. In addition, Fig. 3 shows the TGA curve of the gel after drying at 130°C for 2 days and grinding (DRY). The total weight loss for the DRY

sample is only 5–6% up to 320°C. No weight loss was observed after this temperature. Considering attained information from Fig. 3, 320°C of heat was selected as a suitable temperature for the bioactive gel stability.

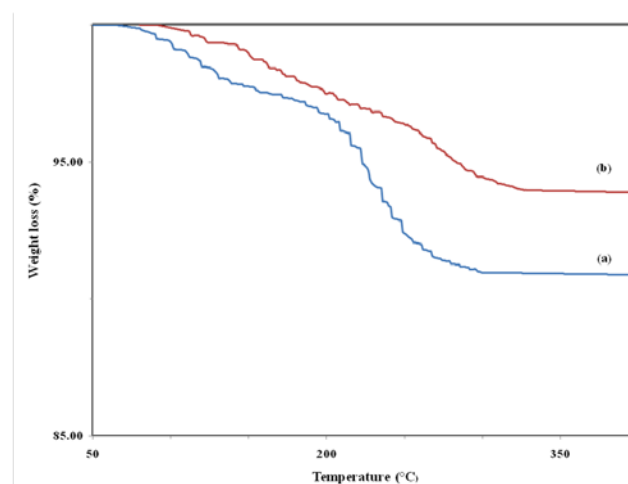


FIG. 3 TGA CURVES FOR WET (A) AND DRY (B) M3 GEL COMPOSITION

### *Surface Characterization*

The XRD study of the monolithic gel glasses confirmed the absence of crystalline phases in the samples, because no diffraction maxima were observed and only a broad band between 18° and 40° 2 $\theta$  was detected for all compositions. The XRD patterns of the surfaces of M3 before and after 14 days immersion in SBF are shown in Fig. 4 as an example. The surface after 14 days immersion showed diffraction peaks corresponding to the (002), (210), and (211) reflections of calcium phosphate carbonate hydroxide [Ca<sub>5</sub>(PO<sub>4</sub>CO<sub>3</sub>)<sub>3</sub>(OH)]. Other less intense peaks of HCA were also identified when the count rate of the XRD analysis was increased to 40 s/step.

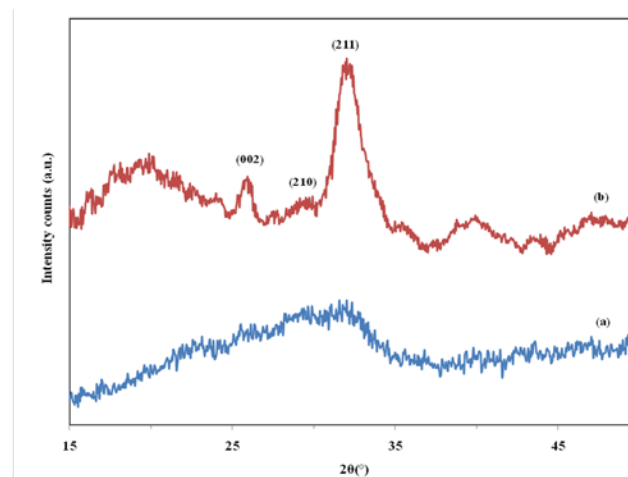


FIG. 4 XRD TRACES OF THE M3 SURFACE BEFORE (A) AND AFTER IN VITRO TESTING FOR 14 DAYS (B). THE STANDARD PEAKS FOR HA ARE IDENTIFIED

Scanning electron microscopy observation of the organic-inorganic composite monoliths showed that the surfaces of all samples were smooth, crack-free and porous. An ideal pore structure was shown clearly, exhibiting a homogeneous interconnected macroporous network with open pores. The diameters of these smaller pores varied from 5 to 10  $\mu\text{m}$ . The Si and Ca content determined by quantitative EDX analysis of the surfaces agreed with the nominal compositions given in Table 1. Figure 5a shows the SEM micrographs of the M3 surfaces before and after immersion in SBF for different periods. After 14 days of immersion, the micrograph (Fig. 5b) indicated the nucleation and growth of spherical particles on the glass surface.

The EDX studies revealed the inclusion of phosphorous on the surfaces of the monolith sample M3. The results showed that the Ca and P concentrations on the surface increased, whereas the silicon concentration decreased. A tiny amount of chlorine ions was also detected on the surface of the SBF-immersed specimen. The Ca/P ratio, determined by semiquantitative EDX analysis of the surface layer after 14 days immersion in SBF, was approximately 1.47 for the M3 sample. This value is comparable to the Ca/P ratio of the human bone, as it ranges between 1.6 and 1.7 depending on the age of the person (Ravaglioli et. al.).

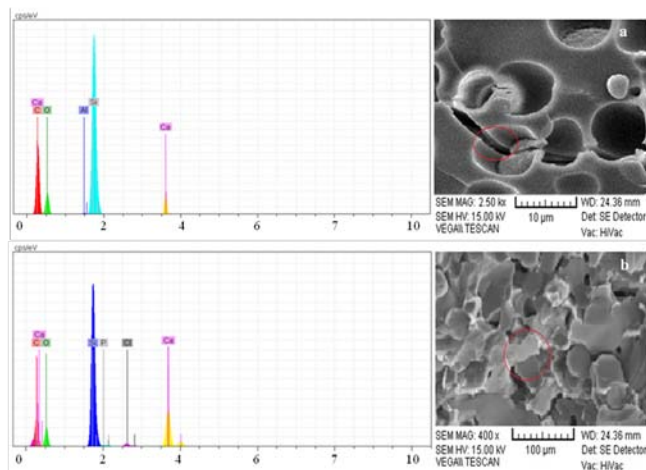


FIG. 5 SEM MICROGRAPH OF M3 SURFACES BEFORE AND AFTER IN VITRO TESTING: (A) PREPERATION, AND AFTER (B) 3, (C) 7 AND (D) 14 IMMERSION IN SBF (ORIGINAL MAGNIFICATION  $\times 2000$ )

### FTIR Investigations on Monolithic Organic-Inorganic Composite

The infrared spectra of all glasses showed an intense band between 1100 and 1040  $\text{cm}^{-1}$ , attributed to an asymmetric vibration of the Si-O-Si bond. The band shown at 814-793  $\text{cm}^{-1}$  corresponds to a symmetric vibration of the Si-O bond. After soaking for 7 days in

SBF (Figure 6b), M3 displayed a band about 600  $\text{cm}^{-1}$  that corresponds to the antisymmetric vibration of the P-O bond in amorphous calcium phosphate. This band evolved after 14 days of assay. Parts b and c of Figure 6, for illustrative purposes, show the evolution of apatite formation on the M3 surface for different assay times.

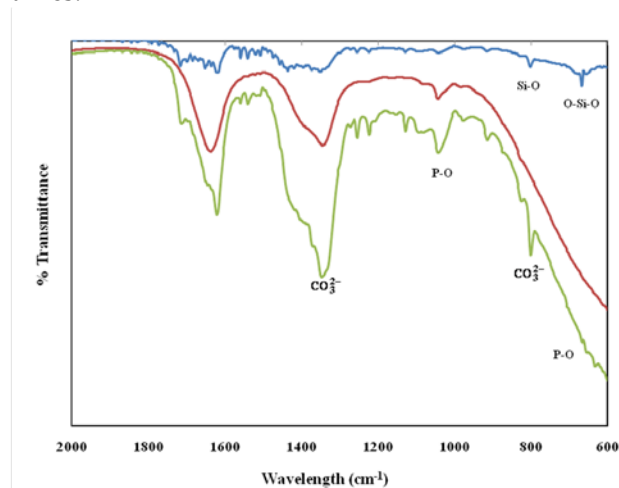


FIG. 6 FTIR SPECTRA OF M3 BEFORE (BLUE) AND AFTER SOAKING FOR 7 (RED) AND 14 DAYS (GREEN) IN SBF

### Conclusions

Polymerized vinyltrimethoxysilane (VTMS), about 20-mers, was mixed with a alcoholic solution of calcium acetate ( $\text{Ca}(\text{OAc})_2$ ) where a typical composition of the mixture was: VTMS: $\text{H}_2\text{O}$ :  $i\text{-PrOH}$ : $\text{Ca}(\text{OAc})_2 = 1 : 6 : 6 : 0.2$  (mol). The resultant sol was heated for 24 h at 60°C to produce a transparent monolithic gel. Then, the wet monoliths were dried to maximum temperature 320°C. The surface analyses showed that the monoliths were all amorphous, homogeneous and porous structure with micron pores; while TGA analyses revealed that residual organic materials, absorbed water and  $\text{Ca}(\text{OAc})_2$  could be removed before 300°C. The in vitro studies showed that all organic-inorganic composite monoliths produced were bioactive. It was concluded that the present monolith was bioactive and can be suitable for the molecular design and fabrication of bioactive multifunctional tissue engineered scaffolds.

### ACKNOWLEDGMENT

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